variance in Ca recovery could be explained by Fe recovery. This data shows that Fe is directly linked to Ca recovery.

Pilot scale and manufacturing scale HTST treatment runs for Media 4 (and variations of Media 4) and Media 9 were also performed. Data was gathered from the runs for analytical assays and for cell culture performance studies to assess the impact of HTST treatment on cell culture performance and product quality. No significant losses of any components were identified from the analytical tests with the exception of iron and copper. In addition, no significant changes in key cell culture performance metrics (titer, growth, viability) or product quality (including basic variants) were observed. Copper loss did not result in changes to cell culture performance or levels of basic variants because the losses were not large enough to illicit changes in the host cell line used for testing based on copper titrations for that cell line. Due to the relatively small amounts of trace metals like iron and copper (75 μM and 1 μM, respectively) compared with calcium and phosphate (1.5 mM and 3 mM, respectively) in the Media 4 formulations it was possible that very small calcium phosphate precipitates ($CaPO_4$ complexes) were formed that did not lead to significant fouling of the heat exchangers but that could interact with the iron and copper present in the liquid medium. These interactions of $CaPO_4$ complexes may chelate the iron and copper in some way as to sequester it from the liquid media and deposit it somewhere within the process flow, along with the $CaPO_4$ complexes that precipitate. Regardless of the mechanism, the fact remains that iron and copper losses are observed upon HTST treatment of media, including when the media is treated successfully to inactivate virus. The iron and copper concentration reductions upon HTST treatment could negatively impact the successful use of the HTST-treated media in the subsequent production phase if the losses are significant relative to the required iron and copper concentrations for the production phase.

Pilot scale and manufacturing scale HTST treatment runs for seven different media formulation were performed. Iron levels in the media formulation were measured pre-HTST treatment and expected levels of iron after HTST treatment were determined. Analysis of iron levels in the media after HTST treatment (post-HTST) demonstrated that HTST treatment resulted in loss of iron levels. Loss of iron was 44% in Media 14, 42% in Media 15, 20% in Media 16, 1.3% in Media 17, and 42% in Media 18 (FIG. **15**). Media 19 and Media 20 were supplemented with iron after HTST treatment.

Growth of NS0 cells, a murine myeloma cell line, in cell culture media that was subjected to HTST treatment and was supplemented with iron was assayed. Analysis of cell growth demonstrated that cells grown in cell culture media not treated by HTST that was either supplemented or not supplemented with iron grew at comparable amounts over time (FIG. **16**). In comparison, cells grew in lower amounts when cultured in media treated with HTST and not supplemented with iron. Addition of iron to HTST treated cell culture allowed recovery of cell growth to higher levels as compared to cell culture media not treated with HTST (FIG. **16**).

Overall, this data demonstrated that Fe losses correlated with calcium, phosphate, and pH levels suggesting a close relationship between trace metal losses and calcium phosphate precipitation events during heat treatment, including those events that are not necessarily detectable as visible precipitates, significant turbidity changes, or operational issues. Loss of trace metals such as Fe can adversely affect cell culture performance in various cell lines and product quality.

What is claimed is:

1. A method for inactivating virus or adventitious agents in mammalian cell culture media while the media maintains suitability for cell culture, said method comprising (a) adjusting pH, limiting the total amount of calcium, and/or limiting the total amount of phosphate in the cell culture media prior to high temperature short time (HTST) treatment such that the formation of complexes comprised of calcium and phosphate is suppressed, and (b) subjecting the cell culture media to HTST treatment to inactivate the virus or adventitious agents, wherein the cell culture media comprises

(i) a calcium concentration of 0 mM to 0.5 mM and a phosphate concentration of 0 mM to 4.5 mM;

(ii) a phosphate concentration of 0 mM to 1 mM and a calcium concentration of 0 mM to 2.75 mM;

(iii) a pH of 5.0 to 6.9 and a total amount of calcium and phosphate of less than 10 mM;

(iv) a pH of 6.4 to 7.4 and a phosphate concentration of 0 mM to 0.5 mM;

(v) a pH of 6.4 to 6.7 and a phosphate concentration of 0 mM to 3 mM;

(vi) a pH of 6.4 to 7.2 and a calcium concentration of 0 mM to 0.5 mM; or

(vii) a pH of 6.4 to 6.7 and a calcium concentration of 0 mM to 1.3 mM.

2. The method of claim **1**, further comprising increasing or limiting the concentration of one or more trace metals in the media prior to HTST treatment.

3. The method of claim **2**, wherein the one or more trace metals is selected from the group consisting of iron and copper.

4. The method of claim **1**, wherein one or more of iron and copper are not present in the media prior to HTST treatment.

5. The method of claim **1**, further comprising supplementing one or more of iron and copper to the media following HTST treatment to a suitable level for cell culture.

6. The method of claim **1**, wherein the pH is adjusted when the media comprises calcium and phosphate.

7. The method of claim **6**, wherein the pH is adjusted in preparing the media prior to HTST treatment to a pH of less than 7.2.

8. The method of claim **6**, wherein the pH is adjusted to less than 7.2.

9. The method of claim **6**, wherein the pH is adjusted to 5.0 to 7.2.

10. The method of claim **6**, further comprising adjusting the pH following HTST treatment to a suitable level for cell culture.

11. The method of claim **10**, wherein the pH is adjusted to 6.9 to 7.2.

12. The method of claim **1**, wherein the total amount of calcium is limited when the media comprises phosphate.

13. The method of claim **12**, wherein the total amount of calcium is limited such that formation of complexes comprised of calcium and phosphate is suppressed.

14. The method of claim **12**, wherein calcium is not present in the media prior to HTST treatment.

15. The method of claim **12**, wherein the pH is adjusted such that formation of complexes comprised of calcium and phosphate is suppressed.

16. The method of claim **12**, further comprising adjusting the calcium level following HTST treatment to a suitable level for cell culture.